AD 674926

TRANSLATION NO. 1305

DATE:

July 68

## DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this document from DIX.

This publication has been translated from the open literature and is available to the general public. Non-DOD agencies may purchase this publication from the Clearinghouse for Federal Scientific and Technical Information, U. S. Department of Commerce, Springfield, Va.

SEP 2 6 1968

20050202041

DEPARTMENT OF THE ARMY Fort Detrick Frederick, Maryland

Reproduced by the CLEARINGHOUSE for Federal Scientific & Technical Information Springfield Va 2151

This document has been approved for public release and sale; its distribution is unlikelied.

Kolosov, S.G. (Professor), and Borisovich, IU. F. (Scientific Collaborator)

Poluchenio sirbiroiazvennykh shtammov dlla tselei vaktsinoprofilaktiki.

Obtainance of anthrax strains for the purpose of prophylactic vaccination

Trudy, Gosudarstvennyl Nauchno-Kontrolinyl Institut Veterinarnykh Proparatov, Ministerstva Seliskogo Khosiaistva SSSR,v.5 (Biopreparaty, vitusy, mikroby). p.5-11. 1955. 41.9 Un39

(In Russian)

Obtainance of anthrax strains for the purpose of prophylactic vaccination

The finding of methods of obtaining anthrax strains with a decreased virulence and with strongly pronounced immunogenic qualities is of considerable theoratical and practical importance.

The method of "directed effectuation" of the micro-organisms is one of the basic methods used in the practice of microbiology for obtaining vaccine strains.

The great Russian scientist I.V. NICHURIN proved by his numerous experiments that living organisms can easily be changed by the experimenter by effectuating their external habitat and also, that there is nothing unchangeable in the world of living organisms.

T.D. LVSENKO, in developing NICHURIN'S teachings, considers that the change of the nature of a living body is the change of the external habitat and of the type of the metabolism. Thus, NICHURIN'S teachings confirm that the nature of living beings - either the most complicated organisms or the lowest [protozoa] micro-organisms - can be changed at the will of the experimenter.

Therefore the method of "directed effectuation" upon the virulent microbes must be used in order to obtain new vaccine strains which possess stable, greatly reduced virulence and immunogenic properties useful for vaccinoprophulaxis.

These two methods of obtaining anthrax vaccines are known in literature. The method of L. PASTEUR and L.S. TSENKOVSKII - by effectuating [subjecting] the virulent anthrax strains to increased temperature - and the method of isolating the noncapsular edemic population on the blood serum (STANATIN, MAC STERNE, and N.N. GINSBURG).

Both methods have enabled the researchers to present effective anthrax vaccines for practical use.

The TSENKOSKII and STI anthrax vaccines are used in practice at the present time for prophylactic inoculations against anthrax. These vaccines have obvious immunogenic qualities and are affective preparations. However, these vaccine can also produce postvaccinal

graph at the work of the second of the

complications.

The experiments of some researchers to improve the TSENKOVSKII vaccine (F.A. TERENT'EV) and to obtain new vaccine (I.F. BATTOK, I.I. ARKHANGEL'SKII, D.R. ROMANOV and others), proved to be unsuccessful. Further experiments were needed for the obtainance of effective and less harmful anthrax vaccines. We chose the method of "directed effectuation" on micro-organisms in order to obtain weak virulent strains good for prophysactic vaccination of agricultural animals against anthrax.

Different methods have been used in our laboratory, such as: the effect of increased temperature, the immune anthrax serum and the method of isolating the population on the congulated serum.

Fifteen strains of virulent anthrax culture were used. This is the history of these strains which succumbed to mutation: strain No. 916 was obtained from the aborted focus of a cow in 1949; strain Sh-2 was isolated from the cadaver of a swine in 1935; strain No. 1260 was separated from the cadaver of a cow in 1949; che standard sporiforous virus was isolated from a cadaver of a horse in 1950; the strains K-5 - was isolated from the cadaver of a cow in 1934 and strain K-12 - from the cadaver of a swine in 1935.

The cultivation on prepared [agar] serums resulted in obtaining the strain 916-1 (KOLESOV, GUTMAN) and the strain Sh-15 (KOLESOV) in 1949. The strain 916-1 possessed the ability to cause edemas in white mice and guinea pigs, seldom in rabbits; it did not cause capsulas. It caused death nearly in all mice and guinea pigs; it did not cause capsulas. The strain caused death in the majority of white mice and - not over 10-20% in guinea pigs. Comparing the obtained and mentioned strains with the vaccine STI - it must be said - that the strain 916-1 has a greater virulence and the strain Sh-15 a less virulence than the vaccine STI. The strains do correspond in their cultural and morphological properties with the anthrax micro-organisms; they formed R-form colonies on MPA and grow like cotton on the bottom of the test tube. The strain 916-1 is able to produce more spores than the strain Sh-15.

The strains 1260-31 and 94 were obtained in 1950-1951 by the method of effectuating the culture of the antianthrax serum. Strain 94 was obtained from a standard sporiforous virus. The strains correspond in their cultural properties with the typical anthrax micro-organism. The strains do not form capsulas. The strain 1206-1 is fully apathogenic to white mice; the strain 94 possesses a weak virulence toward white mice and young guinea pigs; 20-25% of the guinea pigs die. Several strains were obtained by increased temperature; they were of weak virulence. Two of them, namely, strain K-5-39 and strain K-12-46 were studied because of their practical interest. The strains were able to grow on an agar culture medium; by forming. RS-forms, they grow diffusivily and formed moired weaves when shaken. They have a distinctive spore formation, they do not form capsulas. The strains are virulent to white mice and guinea pigs.

The study of the strains which were obtained by the method of "directed effectuation" on the immunogenity on rabbits showed that they do possess distinctive immangenic properties if administered in great doses.

· 1867 - 1975年 - 1986年 - 1986年 - 1986年 - 1986年 - 1986年 - 1987年 - 1986年 - 1987年 - 1987

Control of the immunogenic properties of anthrax cultures

which underwent a directed mutation.

No of	Quentity	Quantity	Ouantity of	Dosta	Conference	Control infaction	70,0	D. C	.
the	of	of	conducted	of	How .	Dosts	חדפת	parvinc	•
Strain	tests	tnooulated	Vaccinations	vaccination	many	of			_
		rabbits	of	<b>I</b> n	days	the virus			**
			rabbits	립	after				-
				Vac	Vaccination				•
1260-31	7	no.	Once	3	28	-1	2	9	1
, 94	1	5	ш	3	25	-	-	7	, ja
R-5-39	2	10		4	71		*		
				1	:	4	7	0	ູ່
R-12-46	2	10	-	Э	34	1	2	8	8
Sh-15		5		,	7.7		}		•
				4	7	4	<b>-</b>	4	္ဌ 、
Control	2	10		-		1	8	7	ŀ

It has been determined that the strain Sh-15 has a more expressed immunogenity then the strains No. 94, 1260-31 and K-5-39, this was obtains after the study of the four strains on the immunogenic properties of sheep in 1951.

Only one sheep from the 8 which were inoculated with the vaccine propared from the strain Sh-15, died four months before control infection. The other 7 sheep showed only a low and short thermal reaction.

Three sheep which were inoculated 15 days before the control infection, survived; they did not show any thermal of general reaction to the fatal doss of the anthrax virus. Four of 5 control sheep which were not vaccinated, died of anthrax.

A careful study in 1951 and 1952 of the vaccine from strain Sh-15 concerning its cultural, morphological and virulent properties showed that the vaccine still retained the properties which it had possessed in 1949 when it was obtained, and also those which it had at the primary tefft - in 1950.

In regard to the cultural morphological properties - the vaccine possesses all characteristics of an anthrax vaccine.

This vaccine - because of its weak virulence - caused death in white mice only when it was administered in massive doses. However guinea pigs remained alive to the greatest extent. The vaccine is harmless to rabbits.

A vaccine, for testing its virulence and immunogenesis, was prepared from the strain Sh-15 in the form of a spore culture fixed in a 20% or 30% (per cant) solution of glycerin at a concentration of 40 to 60 million spores for one ml.

Data obtained from tests of the vaccine for harmlessness in -

2
Ś
9
-
_
5
9

Kind	Quantity Doses	Doses	Namber	Local reaction	action				0.00	Died Committee	
of enfmels		(fn m1)	of test	Any reaction	Size	of the	Size of the reaction	ton tttt		70.10	ę
Rabbits	17 8 8	2 6 4	23	m Î i	9 - 4 - S	44			<b>-</b> •	16 8	94.2
Guin <b>ea</b> pigs	33 4 38	0.0	-0-1-	31.13	6 10 1 1 15 3	1 2 11	31.1			32 4 30	100.0 97.0 100.0 79.0
	<b>7</b> 4		8	4	16 6	13	3		80	34	81.0

The vaccine from strain Sh-15 has been tested for its immunogenesis on rabbits many times in the period of 1951-1952. It was proved every time that the administration optimal doses (1-2ml) guarantees the formation of evident immunity in rabbits. Even small doses (0.3-0.5 ml) produce insusceptibility to anthrax of over 50% in rabbits, at the death of all control animals.

A number of test were recently scheduled for the study of the increase of immnogenic properties of the vaccine prepared from the Sh-15 strain by adding aluminum hydroxide as nonspecific stimulator. It was proved by a number of tests that the aluminum hydroxide increases the immunogenesis of the vaccine.

TABLE No. 3

Data obtained from tests of the vaccine for imminogenetic properties

in rabbits in 1951 - 1952

Glycerinated 2 9 2 1 8 88.8 88.8    vaccine 3 16 1 7 9 56.2    1 3 0.5 1 4 80.0    2 15 0.3 7 8 53.3    Total Clycerinated 2 5 2 2 5 100    vaccine 1 11 1 2 5 100.0    vatch Ao(OH)s    Added    Virus control 2 35 - 88 27 77.1    Vaccine 2 35 - 88 27 77.1	Name of the vaccine	Number of test	Number of rabbits	Dosis in ml	Died	Survived	F2	
rinated 1 45 0.5 1 4 8  rinated 2 5 2 - 5  ne 1 11 1 2 9  14 0.3 6 8  control 2 35 - 8  2 35 - 8  2 37	Glycerinated Vaccine	3 2	9 41	2 1		80 0	88.8	
ringted 1 45 16 29  for a 1 11 1 2 5  for a 2		1 2	3 15	0.3		<b>,</b> 4 ∞	53.3	
ringted 2 5 2 - 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	Total	-4	45		16	29	7.79	
control 1 11 1 2 9  1 3 0.5 - 5  1 4 0.3 6 8  control 2 35 - 8  2 35 - 8  2 37  8 27	<b>Glycerinated</b>	7	. 5	2	•	<b>'</b>	100	
control 2 35 - 5 5 5 6 8 8 control 2 35 - 8 8 - 5	vaccine	<b></b> 1 :	11	<b>;1</b>	7	6	81.8	
ontrol 2 35 - 8 27 8 - 8	<b>v1</b> th Ao (OH) s	7	e 4	0 5 6	l ve	vΩ	100.0	
2 35 - 8 27 8 8 -	added			2		0	۲۰/۲	<b>d</b> ere
	Virus control	7	32 8	1	80 80	27	77.1	

Positive results have been obtained from the vaccination of agricultural animals in 1951 and 1952 testing the vaccine from the strain Sh-15 for harmlessness.

Thus, there were vaccinated in the second half of November 1951 eleven horses with 1 ml and one foal with a dosis of 0.3 ml, nine cows with a dosis of 1 ml and three calves with - 0.3 ml, twenty sheep with - 0.2 ml and ten lambs with a dosis of 0.1 ml.

The local reaction to the inoculated vaccine was limited and not strong, the temperature of the body increased 0.5 - 1 only and lasted just for a short time (1-2 days).

The harmlessness of the vaccine also has been tested and proved on young goats and sheep in October 1952.

The vaccine used in the vaccination of goats was either glycerinated vaccine was administered vaccine with aluminum hydroxide added. The vaccine was administered in the usual dosis (0.2 ml) or in doses which were 2-3 times or even 4 times greater. There were 33 goats vaccinated. It should be mentioned that the vaccination took place in October, at an unfavorable, rainy season. However, notwithstanding these circumstances, the vaccination was a success, since the local reaction in the animals was insignificant and the temperature, which had risen 0.4 - 1.4 only, lasted just 1-2 days. From all the vaccinated goats just one little goat had a high temperature. Thirty six sheep were vaccinated with the same vaccine, at the same time, among them ten 2-3 months old lambs.

The sheep were vaccinated with a 0.2 ml dosis. These vaccinations did not have any complications either; the local reaction was insignificant, the temperature rise was of short duration.

Thus, the preliminary tests have proved that the vaccine is harmless if inoculated to young goats and sheep.

## Conclusion

- 1. The new anthrax vaccine obtained by "directed effectuation" and selection proved to be harmless and immunogenic, when tested on rabbits.
- 2. The same vaccine, tested for harmlessness on sheep and goats in the conditions of the Institute, showed that it is harmless and stimulates just a manor local and thermal reaction.
- 3. It was determined that the vaccine produces stable immunity at a control after 15 days and after 4 months subsequent inoculation; this was proved by testing the vaccins for stability and length of the immunity period on sheep.
- 4. Further studies of the vaccine should be conducted in testing its harmlessness and effectiveness on agricultural animals at large and in rigorous experiments for the lingth and stability of its produced immunity.

## Literature

ARKHANGEL'SEIX, I.I. and ANTONOV, V.K. Biological changes in B. anthragis under the imfluence of some physical factors. "Zhurnal mikrobiologii, epidemiologii i immunobiologii" No. 11, 1948.

RATIUK, I.' F. Werkering of Ensithracis under the influence of X rays in order to obtain stable vaccines. "Zhurnal mikrobiologii, epidemiologii i immunobiologii" No. i v. XVIII, 1957.

GAMALEIA, N.F. Prophylactic vaccination against anthrax. Reminiscences. Izd-vo Akademil Nauk SSSR, 1947. GRINBALM, F.T. The problem of microbe mutation. Trudy Konforentall Institutov Epidemiologii, i Mikrobiologii po problems immontohivosti Mikrobev. [Treatise of the Conference of the Institutes of Epidemiology and Microbiology on Problems of Microbe Mutation] Cor'kii, 1949. KALINA, G.P Mutation of pathogenic micro-organisms. Gosmedizdat USSR. Kiev, 1949. GINSBURG, N.N. Anthrax vaccine STI. "Veterinariia" No.11, KOLKSOV, S.G. The Vaccines against anthrax. "Biologicheskio 1. chimlotarapovtichskie veterinarnye preparaty" Sclikhosgiz, 1948. KOLESOV, S.G. The contemparary state of matrixes of the Tsenkovskii anthrax vaccine. "Sibirakaia iazva" Scl'khosgiz, 1940. KIRSANOV,  $V_{\bullet}K_{\bullet}$  On the question pleomorphism in anthrax micro-organism. Trudy Gosudarstvennogo Institua Eksperimental nol veterinarii, v.2, issue 1, 1924. LYSENKO, T.D. News in science on the biological look [type]. Doklady Vseseiuxnoi Adademil Sel'skokhoziatatvennykh Nauk imenl Leniza [Reports of the all-Union Agicultural Academy named after Lenin, issue 12.1950. MICHURIN, I.V. Compositions. v. IV. Sel'khozgia, 1948. TERENT'EV, F.A. Morphological Changes in B. anthracis. "Shirakaia iazva" Sel'khozgiz, 1940. TSENKOVEKII, L.S. On Pasteur's vaccinations, Trudy Vol'noge Ekonomicheckogo Obshohestvs [Treatise of the Liberal Economical Society], 1883-1885. SNALASHNIKOV, A. A sketch of Prof. Tsenkovskii's works on prophylactic vaccination against anthrax. Sbornik trudov Khar kovokogo Veterinarnago Instituta, [Collection of works of the khar'kov Veterinary Institute], v.2, 1888.

AMV/JA